

# Notch and presenilins in vertebrates and invertebrates: implications for neuronal development and degeneration

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Recent progress in elucidating the biology of Notch and presenilin has revealed a close functional relationship between these two proteins during cell fate determination in worms, flies and humans. Presenilins are required for the putatively intramembranous proteolysis of Notch to release its intracellular domain to the nucleus. This finding establishes a specific biochemical role for presenilins in Notch signaling and interfaces with emerging evidence about how *frizzled*, *disheveled* and numerous other genes regulate the highly complex Notch pathway. Advances in understanding Notch and presenilin functions in the differentiation of neurons and non-neural cells have important implications not only for development but also for late-life degenerative disorders such as Alzheimer's disease.

## Addresses

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## Abbreviations

A $\beta$	amyloid $\beta$ -protein
AC	anchor cell
AD	Alzheimer's disease
APP	$\beta$ -amyloid precursor protein
CSL	CBF1/Su(H)/LAG-1
DSL	Delta/Serrate/LAG-2
LNG	LIN-12/Notch/GLP
NICD	Notch intracellular domain
PS	presenilin
TM	transmembrane domain
VPC	vulval precursor cell
VU	ventral uterine precursor cell

## Introduction

Biologists studying the structure and function of genes and their products in invertebrates have increasingly become interested in the implication of some of these proteins in hitherto obscure human diseases. In turn, students of human pathology have provided insights relevant to the most fundamental of cellular processes. This blurring of

basic and applied research is particularly well exemplified by progress in understanding the biology of the Notch family of receptors, which plays a central role in cell fate determination during development in multicellular organisms. In the title of this review, I have linked two proteins, Notch and presenilin, and two seemingly disparate processes, early development and late-life neurodegeneration. During the past 18 months, new evidence of a specific functional relationship between these two proteins and their implication in both neurodevelopment and neurodegeneration have emerged. Although this forum requires a highly selective review of this burgeoning literature, the examples cited should amply demonstrate the dynamic progress in this aspect of signal transduction and the potentially profound clinical implications that derive from it.

Signaling mediated by the cell surface receptors Notch in *Drosophila* and LIN-12 and GLP-1 in *Caenorhabditis elegans* has been shown to be essential for a large variety of cell fate decisions during development (for general reviews, see [1–3]. These three members of the LIN-12/Notch/GLP (LNG) receptor family share a domain architecture and have a similar signaling pathway. Activation of the LNG receptors requires the DSL (Delta/Serrate/LAG-2) family of cell surface ligands; an activated receptor is then linked to its transcriptional response by the CSL (CBF1/Su(H)/LAG-1) family of DNA-binding proteins (Table 1).

Two general types of LNG-mediated signaling are recognized: inductive signaling, in which neighboring but nonequivalent cells express either a DSL ligand protein or an LNG receptor; and lateral signaling, in which initially equivalent neighboring cells undergo a transformation to nonequivalent cells that express either ligand or receptor. An example of inductive signaling involving the LIN-12/Notch pathway occurs when a somatic gonadal cell in *C. elegans* (containing a DSL ligand such as LAG-2) signals a neighboring germline cell (containing the receptor GLP-1) to divide mitotically, thus maintaining a population of germline stem cells in the adult [4]. Inductive signaling is also responsible for establishment of the dorsal–ventral cell pattern at the wing margin of *Drosophila*;

Table 1

Nomenclature of some of the principal protein components of the Notch signaling pathway.

Organism	Ligands (DSL family)	Receptors (LNG family)	Downstream effectors (CSL family)
<i>C. elegans</i>	LAG-2, APX-1	LIN-12, GLP-1	LAG-1, EMB-5
<i>Drosophila</i>	Delta, Serrate	Notch	Su(H)
Mammals	Delta 1, 2 Jagged 1, 2	Notch1–Notch4	CBF1 (RBP-Jk)

dorsal cells signal with the DSL ligand Serrate, whereas ventral cells use Delta for this purpose, and both cell types receive their respective signals using Notch [5]. Examples of lateral signaling via the LIN-12/Notch pathway include the specification of neural versus non-neural cell types in embryos (e.g. the neurons versus epidermis decision), specification of sensory organ precursor cells in fly bristles, and specification of R8 photo receptors in the eye of *Drosophila* (reviewed in [1]).

### Probing the biochemical mechanisms of Notch activation

The vital importance of cell-cell interactions controlled by the LIN-12/Notch pathway for proper development of invertebrates and vertebrates is clear from many genetic analyses but the precise biochemical mechanism by which these receptors transmit cell surface signals to the nucleus to alter expression of a variety of genes has been poorly understood. Among the most exciting developments during the ~18 month period under review has been the publication of a number of reports that shed considerable new light on how the Notch receptor operates.

Even before the current work, it appeared that signal transduction by ligand-activated Notch might require proteolytic processing to release the Notch intracellular domain (NICD) to the nucleus (e.g. [6]). In an extension of this hypothesis, Schroeter *et al.* [7\*\*] identified and mutated a cleavage site within or just cytoplasmic to the single transmembrane domain (TM) of Notch. The mutation markedly decreased Notch signaling in mammalian cells by inhibiting Notch cleavage, thus clearly linking intracellular proteolysis of Notch with its function in activating transcription of nuclear genes. A principal concern about the 'proteolytic hypothesis' of Notch signaling was the inability to detect the NICD in the nucleus. This was shown to be a result of very low nuclear concentrations of NICD, sufficient to regulate gene transcription but too low to be detected by conventional immunocytochemistry. However, cells transfected with a full-length Notch-1 construct and then stimulated by the natural DSL ligand Jagged-1 produced the cleaved NICD product, which could be recovered as a complex with the effector protein, CSLrbp [7\*\*]. Three other groups came to similar conclusions in *Drosophila* using different approaches to show Delta-induced release of the NICD to the nucleus [8\*-10\*]. Thus, binding of mature Notch by ligand at the cell surface induces apparent intramembranous proteolysis of Notch and nuclear entry of the cytoplasmic domain, to which there binds and activates the CSL family of DNA-binding proteins.

An essential question raised by these new findings regards the nature of this highly unusual scission. An answer — at least a partial one — has not been long in coming. Four papers published simultaneously in *Nature* provide compelling evidence that the presenilin proteins are essential participants in this cleavage event [11\*\*-14\*\*]. The homologous mammalian proteins presenilin 1 (PS1) and

presenilin 2 (PS2) were identified in 1995 as the genes responsible for a substantial fraction of the early onset, autosomal-dominant form of Alzheimer's disease (AD) [15,16]. Shortly thereafter, a nematode homologue of the presenilins, SEL-12, was identified by genetic methods and shown to facilitate signaling by LIN-12, a Notch receptor in worms [17]. Meanwhile, investigators examining the molecular mechanism of AD caused by mutant presenilin showed in cultured cells, transgenic mice and AD patients themselves that missense mutations in the PS1 and PS2 genes lead to a selective increase in the cleavage of the  $\beta$ -amyloid precursor protein (APP) at residue 42 of its amyloid  $\beta$ -protein ( $A\beta$ ) region (reviewed in [18]). The unknown protease(s) that effects both this cleavage and another just two residues amino-terminal to it (producing the  $A\beta_{40}$  fragment of APP) have been dubbed  $\gamma$ -secretase(s) and these have been hypothesized to be unusual aspartyl protease(s) that can cleave substrates within the membrane [19\*]. While the normal functions of APP remain unclear, its constitutive cleavage by the recently identified  $\beta$ -secretase (a single transmembrane aspartyl protease with its own active site in its ectodomain [20-22]) and then by  $\gamma$ -secretase releases the  $A\beta_{42}$  and  $A\beta_{40}$  peptides that accumulate to potentially toxic levels in brain regions important for memory and cognition in all cases of AD [18].

Two of the four recent papers in *Nature* [13\*\*,14\*\*] show that lethal loss-of-function mutations in *Drosophila* presenilin abolish Notch signaling by preventing NICD from being released to the nucleus. The PS null mutations produced a somatic and neural phenotype that appeared highly similar to that of embryos lacking *Notch*; however, one of these papers implicated presenilin in the final, putatively intramembranous cleavage of Notch [13\*\*], whereas the other provided evidence that presenilin's role was upstream of this event [14\*\*]. In the third paper of the *Nature* quartet, De Strooper and colleagues [12\*\*] followed up on their earlier demonstration [23\*\*] of an essential requirement for PS1 in the  $\gamma$ -secretase-mediated cleavage of APP by showing that mouse cells devoid of PS1 undergo markedly decreased proteolytic release of NICD from a Notch construct. A similar conclusion was reached recently by Song *et al.* [24\*], but these workers additionally reported that AD-causing missense mutations in human PS1 also impair this processing. The latter result is surprising, as previous data suggested that such mutations involve gain rather than loss of mammalian presenilin function [25,26]. De Strooper *et al.* [12\*\*] also reported that peptidomimetic compounds designed to inhibit the  $\gamma$ -secretase processing of APP showed the same rank order of potency in decreasing the intramembranous cleavage of Notch.

Taken together, the papers just summarized provide compelling evidence that presenilin plays an essential role in the intramembranous proteolysis of both Notch and APP (Figure 1). But how does it do so? A provocative explanation is suggested by the fourth *Nature* paper [11\*\*]:

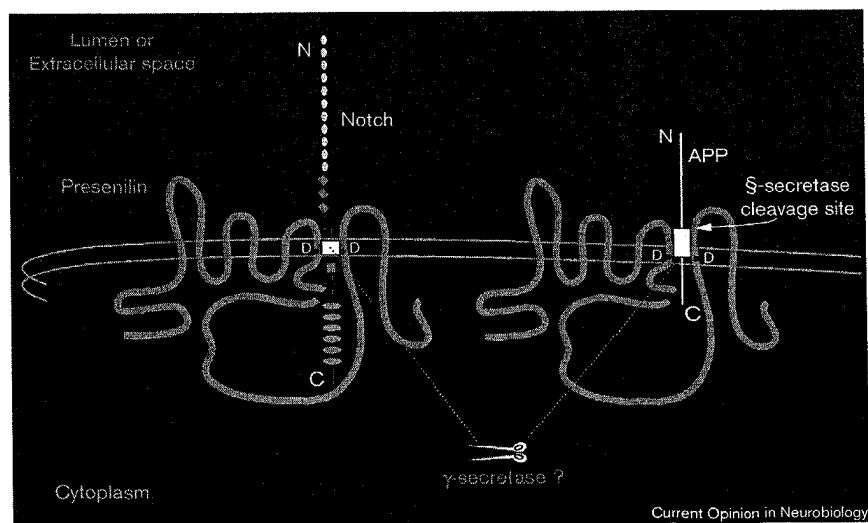
presenilins might themselves be the long-sought  $\gamma$ -secretase(s). Recent work by Li and Greenwald [27\*] had provided confirmatory evidence that an eight TM domain model of PS topology is most plausible, placing the amino and carboxyl termini and the large 'loop' between TM6 and TM7 in the cytoplasm. Close inspection of the PS1 sequence led Wolfe *et al.* [11\*\*] to notice two aspartates located in analogous positions near the middle of TM6 and TM7, respectively, and these were experimentally mutated to alanines. Either mutation, when expressed in various mammalian cell types, prevented both the normal endoproteolysis of PS1 within the hydrophobic region of the TM6-TM7 loop (a cleavage that appears to be required for functional activity of wildtype PS) and markedly inhibited the  $\gamma$ -secretase cleavage of the 99-residue carboxy-terminal fragment of APP (C99), thereby lowering levels of  $A\beta_{40}$  and  $A\beta_{42}$ .

It had been shown previously that steady-state levels of presenilin are tightly regulated by limiting cellular factors that allow only a minority of overexpressed full-length PS molecules to undergo endoproteolysis and stabilization of the resultant heterodimeric complexes of amino- and carboxyl-terminal PS fragments [28,29], with excess holoprotein undergoing rapid degradation, in part by the proteasome [30]. The aspartate-mutant forms of PS1 remained uncleaved and thus nonfunctional but they largely replaced the endogenous PS heterodimers, thus acting in a dominant-negative fashion [11\*\*]. Conservative substitution of aspartate by glutamate still abrogated the

$\gamma$ -secretase cleavage of APP, indicating a specific requirement for the two TM aspartates. These results are consistent with one of two mechanisms: a role for presenilin as a unique 'diaspartyl' cofactor for  $\gamma$ -secretase, or its actual function as  $\gamma$ -secretase, an unprecedented intramembranous aspartyl protease. An observation favoring the latter hypothesis was the *de novo* generation of  $A\beta$  at mildly acidic but not at neutral pH in a microsomal transcription/translation reaction [11\*\*], consistent with the requirement of an aspartyl protease to have one protonated aspartate (discussed further in [19\*]). The residual  $\gamma$ -secretase activity observed in the PS1 aspartate mutant transfectants has subsequently been shown to be due to endogenous PS2 by mutating its TM6 and TM7 aspartates (these residues are conserved in all presenilins) [31]. Indeed, stable expression of TM aspartate $\rightarrow$ alanine mutations in PS1 and PS2 in the same cells dropped  $A\beta$  levels to undetectable, suggesting an absolute requirement for functional presenilins (and their TM aspartates) to generate any  $A\beta$  [32].

The hypothesis that presenilins are  $\gamma$ -secretases predicts two results: these are that at least some protease inhibitors designed to block  $\gamma$ -secretase will turn out to bind directly to presenilin, and that  $A\beta$  will be able to be generated by presenilin from C99 in pure lipid vesicles. The latter experiment is needed for absolute confirmation but it will be difficult to accomplish as it is already clear that other cellular proteins are required for proper PS endoproteolysis and fragment stabilization [28,29]. Mutagenesis studies suggest that

Figure 1



Hypothetical model of the role of presenilin (PS) in Notch and APP processing based on current information. The diagram shows the predicted 8 TM domain topology of PS, which occurs principally as a cleaved heterodimer. Some Notch and APP molecules form complexes with PS. Two aspartates (D) in TM6 and TM7 of PS are required for the cleavages of Notch and APP within their TM domains, and these may align with the respective sites of cleavage in the two substrates. It is unknown whether PS directly effects these cleavages or whether a still unidentified aspartyl protease ( $\gamma$ -secretase) present in the complexes does so. Several motifs are depicted in Notch: EGF-like repeats (yellow circles), LNG repeats (orange diamonds), a single TM domain (white box), the RAM23 domain (blue square), a nuclear localization sequence (red rectangle), and six cdc10/ankyrin repeats (green ovals). Following the putative intramembranous cleavage mediated by PS, the Notch intracellular domain is released to the nucleus to activate transcription of target genes. APP contains the  $A\beta$  region (white box), which is released into the lumen after sequential cleavages of APP by  $\beta$ -secretase and then  $\gamma$ -secretase/PS. The fate of the APP intracellular domain is unknown.

it is not the large cytoplasmic loop but rather the extreme carboxyl terminus of PS that could turn out to be the site for binding of these limiting factors [33\*,34]. The notion of a direct role for presenilins in catalytic complexes that include Notch and APP as substrates is consistent with earlier work reporting co-immunoprecipitation of full-length APP and presenilins [35,36]; moreover, similar complex formation between Notch and PS has recently been observed [37]. An alternative view — that PS and APP do not associate [38] and that instead, presenilin is a regulator of membrane-trafficking that brings components of the proteolytic reaction (APP, Notch and  $\gamma$ -secretase) together — has been put forward ([39\*]; Figure 2).

Regardless of which functional model is correct — trafficking or catalytic complex formation — it becomes increasingly important to resolve an additional controversy. In precisely which membranous compartments does presenilin occur? Because Notch and APP are cell surface proteins and Notch in particular requires activation by a DSL ligand from adjacent cells, one would expect presenilin to be found at or near the plasma membrane. In point of fact, *Drosophila* presenilin has been reported to be detected in or very near the plasma membrane [40]. Although most localization studies of the mammalian protein have reported solely endoplasmic reticulum, intermediate compartment and early Golgi loci (e.g. [41,42]), very recent studies provide convincing evidence that some mammalian PS1 can be found at the cell surface (where it can be biotinylated) and small amounts can complex with mature Notch [43,44\*]. Considerable indirect evidence suggests that surface APP can be

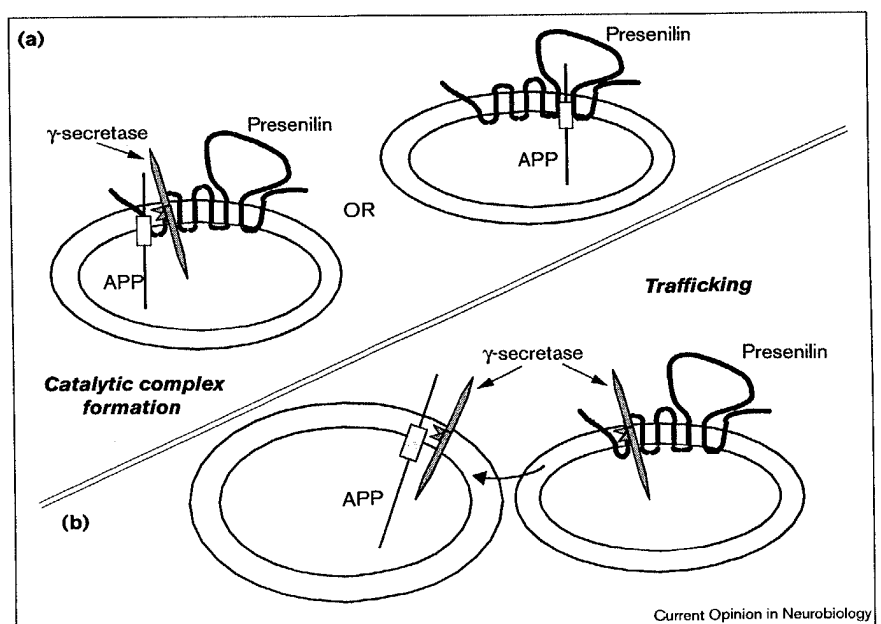
processed to A $\beta$  by  $\gamma$ -secretase at least in part within recycling endosomes (e.g. [45]; for a review, see [18]). The new findings that some presenilin heterodimers reach the plasma membrane is consistent with its role in ligand-activated proteolytic processing of Notch during cell fate decisions.

### Notch uses multiple complex strategies to regulate cell fate determination

The great variety of cell lineage decisions involving Notch predicts that highly heterogeneous strategies for initiating and regulating these different intercellular signaling events exist. This complexity is exemplified by the ongoing examination of the role of the Notch homolog LIN-12 in different cell fate decisions during vulval development in *C. elegans*. The 'anchor cell' (AC)/ventral uterine precursor cell (VU) decision has been shown to involve a pattern of LIN-12 accumulation that directly reflects the pattern of *lin-12* transcription: LIN-12 is initially present in both cells but disappears from the presumptive AC and is then restricted to the presumptive VU [46]. Thus, activation of *lin-12* results in the VU fate; failure to activate *lin-12* results in the AC fate. In contrast, the specification of vulval precursor cells (VPC) is more complicated; here the pattern of LIN-12 accumulation does not simply reflect the pattern of *lin-12* gene expression. Instead, LIN-12 accumulation is reduced specifically in just one of six VPCs in response to an epidermal growth factor (EGF)-like inductive signal from the AC that involves activation of the Ras pathway [46]. These data confirm the existence of entirely distinct mechanisms by which LIN-12 can mediate cell fate during development of one organism,

**Figure 2**

Two alternative models for the function of presenilins in the  $\gamma$ -secretase-mediated intramembranous proteolysis of APP. In (a), presenilin (shown in its postulated 8 TM topology) interacts with the substrate APP and acts as a direct catalytic cofactor [11\*,36]. In (b), presenilin and APP do not interact directly; rather, presenilin serves to traffic either  $\gamma$ -secretase or APP to the other within a membranous microdomain in order to allow later proteolysis to occur [38,39\*]. The box in the APP diagram represents the 40–42 residue A $\beta$  region of the precursor.



including the response of some cells to an inductive signaling pathway.

Another intriguing example of the complex regulation of Notch signaling arises from recent studies of the R3 and R4 photoreceptors in each ommatidium of the *Drosophila* eye [47<sup>\*\*</sup>,48<sup>\*\*</sup>]. The genes *frizzled* (*fz*) and *disheveled* (*dsh*), which encode components of a signaling pathway required in the R3 photoreceptor, seem to promote activity of the Notch ligand, Delta, and thus inhibit Notch receptor activity in R3, so that Notch becomes activated selectively in adjacent R4 cells. It thus appears that small differences in the timing or level of *fz* activation between R3 and R4 produce a binary fate decision between these two cells. Subsequent feedback in the Notch pathway allows this difference to become amplified, as has been previously shown in other Notch-mediated cell fate decisions. These results place *fz* (and *dsh*) as upstream regulators that can promote Delta activity and inhibit Notch in a coordinated manner. Such polarized Notch activation in response to different signaling pathways probably plays a role in other precise positioning events of neural cells within clusters of proneural precursors, and various neural and non-neural systems are likely to use analogous developmental mechanisms (e.g. [49]).

It is well known that the principal components of the Notch signaling pathways are highly conserved throughout evolution. The cloning of the human homologue (DTX1) of the fly *delta* gene, a positive regulator of the Notch pathway, provides another example of this conservation [50]. Both molecules bind to the ankyrin/cdc10 repeats in the Notch cytoplasmic domain and thereby help modulate the activity of Notch-dependent basic helix-loop-helix transcription factors.

### Importance of Notch signaling for cell differentiation in the nervous system

Although great progress has been made in elucidating the functions of Notch during early development of invertebrates, there is also growing recognition of the importance of Notch signaling in the establishment of cell identity and differentiation in mammals, not only during development but also in adult life. For example, Notch genes are expressed in adult human hematopoietic progenitor cells and mediate the differentiation of specific myeloid progenitors into granulocytes in response to different types of cytokines [51<sup>\*</sup>]. As in other systems, persistent high levels of expression of Notch receptor genes allows hematopoietic cells to remain multipotent and to be able to subsequently adopt alternative cell fates. The importance of lateral signaling through the LIN-12/Notch pathway for regulating neural cell specification has long been known, and intriguing new information about this role of Notch has recently emerged. In cultured mouse cortical neurons, contact-mediated Notch signaling has now been shown to regulate the ability to extend and elaborate neurites [52<sup>\*\*</sup>]. High levels of Notch activity coincide with an increase in the number of

interneuronal contacts and the cessation of neurite growth. In contrast, low levels of Notch activity are associated with neuritic extension; subsequent upregulation of Notch either inhibits extension or actually causes retraction of neurites. Thus, as in many other cell populations, Notch inhibition in neurons promotes a differentiated phenotype, whereas Notch activation leads to an arrest of the neuron's ability to extend and elaborate neurites. Given the role of presenilin in mediating the final proteolysis of Notch to allow its nuclear signaling (see above), it is not surprising that PS1-deficient mice were previously found to have specific neurodevelopmental abnormalities in the periventricular proliferative zone of the forebrain [53]. Similarly, mutations in *Drosophila* presenilin lead to several developmental defects, including incomplete neuronal differentiation within the larval CNS, where Notch heterodimers accumulate on the surface of neuroblasts [54].

In addition to the requirement for Notch and presenilin during both neuronal specification and neurite outgrowth in invertebrates and mammals, Notch activation by the ligand Jagged-1 has recently been found to markedly inhibit differentiation of oligodendrocytes [55]. These results raise the prospect that the timing of myelination and perhaps its localization in the developing CNS is controlled in part by the Notch pathway.

### Progress in Notch and presenilin biology has mechanistic and therapeutic implications for human disease

Although the hypothesis that A $\beta$  accumulation in brain regions important for memory and cognition is a necessary initiator of Alzheimer's disease remains to be proven, enough evidence exists to make inhibition of A $\beta$  production an attractive therapeutic strategy [18]. Exciting new developments summarized above suggest that presenilin could be an appropriate target for small inhibitory compounds, regardless of whether it serves as  $\gamma$ -secretase or an essential cofactor thereof. We should soon know whether existing or newly designed inhibitors that block A $\beta$  production at the level of  $\gamma$ -secretase can actually bind and inactivate presenilins. If so, such compounds would be expected to interfere to some extent with Notch intramembranous cleavage, an event that is crucial for Notch signaling in both mitotic and post-mitotic cells of mammals. But even if such functional activities in adult humans are clinically important, the finding that amounts of NICD so low as to be virtually undetectable can still effect signaling [7<sup>\*\*</sup>] suggests that partial inhibition (e.g. by 30–40%) of presenilin and/or other components of the  $\gamma$ -secretase mechanism might be achieved without major untoward consequences. There are notable precedents for partially inhibiting crucial enzymes in humans (e.g. HMG CoA reductase; angiotensin converting enzyme) without incurring unacceptable cytotoxicity. Because small molecules that inhibit the  $\gamma$ -secretase processing of APP are approaching clinical evaluation, their therapeutic windows will soon become known.

The remarkable progress in Notch and presenilin biology during the brief period under review allows one to place the emerging public health catastrophe of Alzheimer's disease into a new perspective. It might turn out that the principal conserved function of presenilins is to mediate the proteolytic processing of the Notch receptors, thereby conferring great developmental advantages during evolution. However, the survival of large numbers of humans far beyond reproductive age as a result of advances such as antibiotics in the last century may have increasingly permitted a kinetically less favored substrate of this reaction (APP) to be converted to a highly stable, long-lived product ( $A\beta_{42}$ ) that can gradually accumulate to produce progressive neurodegeneration. Quantitative biochemical comparisons of Notch and APP as binding partners and substrates of presenilin would help support or deny this hypothesis. I would even speculate that partial loss of function mutations in human PS1 could decrease the efficiency of APP processing to  $A\beta_{42}$  (just as AD-causing gain of function mutations increase it), and that such mutations might be found in very old humans (e.g. centenarians) who have experienced little age-related  $A\beta$  accumulation and thus escaped AD. In any event, it now appears that Alzheimer's disease can be added to the previous examples of devastating human disorders, such as CADASIL [56], Alagille syndrome [57,58] and neoplastic transformation [59], that appear to involve changes in the Notch signaling system.

### Acknowledgements

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